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Research Article

Evaluation of Efficiency of Pretreatment Methods on Corncob for Bioethanol Production

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Abstract

The pre-treatment of feedstock is an integral step introduced for the degradation of the lignocelluloses components. However, pre-treatment methods can be used on various types of feedstock such as sucrose, starch, lignocelluloses and algal biomass through fermentation process by microorganisms. Compared to other types of microorganisms, yeasts especially *Saccharomyces cerevisiae* and *Aspergillus niger* are the common microbes employed in ethanol production due to its high ethanol productivity, high ethanol tolerance and ability of fermenting wide range of sugars to ethanol. This work was aim at comparing the efficiency of pretreatment methods in the production of bioethanol from corn cob. Corncob sample was grinded and exposed to steam explosion and dilute acid pretreatment, *Saccharomyces cerevisiae* strains were screened based on ethanol tolerances. Therefore, the bioethanol concentration was determined using specific gravity test. After analyzing the result statistically, the result obtained showed that dilute acid pretreatment is significantly different with p-value 0.02 ($p<0.05$). Among the pretreatment methods used dilute-acid pretreatment yielded the highest concentration of 25.03% (w/v), steam explosion 15.99% (w/v) and finally physical pretreatment with the least 5.99% (w/v).

Keywords: Pretreatment; Lignocelluloses; Feedstock; Bioethanol; Corncob.

Introduction

Production of bio-ethanol from lignocelluloses materials such as agricultural wastes though faces challenges, can substitute bio-ethanol production from edible food substances. Maize (*Zea mays*) is the most abundant cereal produced in Nigeria. This is accompanied by large quantities of maize agro wastes which is underutilized. Corncobs form about 30% of maize agro-wastes [1]. Currently the corncobs are burnt as fuel in households of peasant rural farmers. Production of bio-ethanol from maize agro waste has been attempted with enzymes from different sources for hydrolysis of lignocelluloses and with different organisms for fermentation [2].

Bioethanol as one of the biofuel has been applied in automobiles with gasoline in different blending proportions [3].

One of the greatest challenges of the twenty-first century is to meet the growing demand of energy for transportation, heating and industrial processes, and to provide raw materials for chemical industries in sustainable ways. At the beginning of the 20th century, many industrial materials such as dyes, solvents, and synthetic fibres were made from trees and agricultural crops. By the late 1960's, however, many of these bio-based chemical products were replaced by petroleum derivatives which could be produced at lower cost [4].

Over the last few decades, the negative impacts of fossil fuel on the environment and

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consequent global warming, progressive demand for energy, inevitable depletion of the world's energy supply, and the unstable oil market (such as the energy crisis of the 1970s) have renewed the interest of society in searching for alternative fuels [5]. The alternative fuels are expected to satisfy several requirements including substantial reduction of greenhouse gas emission, worldwide availability of raw materials, and capability of being produced from renewable feedstocks.

Microorganisms play a significant role in production of bioethanol from renewable resources and thus, selection of suitable strain is essential for the individual process. Wide varieties of microorganisms including yeasts, bacteria and fungi have been exploited offering different advantages and disadvantages. However, the most frequently used microbe has been yeast and among the yeasts, *Saccharomyces cerevisiae*is the most preferred strain since it can tolerate ethanol concentration as high as 20% in the fermentation medium [3].

Nigeria produces large quantity of lignocelluloses wastes like corn cob, saw dust, sugar cane bagasse, rice husk and switch grass. Most of these wastes end up in the environment thereby constituting environmental pollution problem instead of serving as a source of renewable energy. Challenges in the identifications of the most promising pre-treatment methods used in the breaking down of this lignocelluloses compound are the most difficult aspect to be undertaken due to the structural and compositions of different lignocelluloses compound. Various studies and research have shown that these residues can be biologically exploited for the synthesis of chemicals and fuels [6,7].

The largest environmental issue of our time is energy and waste. Energy extraction accompanied by environmentally safe disposable of a cellulosic waste will render the process economically feasible. Processing such wastes for ethanol fermentation is highly appealing to reduce the pollutants [8]. Pre-treatment of a feedstock is an integral step in bioethanol production since various pre-treatment options exist; a study that compares their efficiency in bioethanol production is of importance and it is accepted that renewable energy will be more reliable than current energy resources, therefore there is need to started to develop alternative energy sources to support the world's energy consumption needs and to decrease environmental pressures [1].

The present work was aimed at comparing the efficiency of pretreatment methods in the production of bioethanol from corn cob. The aim can be achieved through the isolation *Aspergillus niger* and *Saccharomyces cerevisiae* from soil and local wine, the screening of *S. cerevisiae* isolates for alcohol tolerance (ethanol tolerance), the determination of the OH groups in the fermented hydrolysates and the determination of the concentration of the ethanol produced.

Materials and methods

Study area and sample collection

The study was conducted at National Research Institute for Chemical Technology (NARICT) Sabon Gari, Kaduna State and the samples were collected from roasted corn sellers in Sabon Gari market, Zaria and humus soil sample was collected from a dumpsite in Dogarawa, Zaria where 30 g soil from 5 cm the top of the soil profile using a hand trowel into a sterile polyethene bag while Fermented burukutu was collected in a sterile container from a supplier in Sabon Gari, Zaria. They were transported to the Environmental Industrial Technology Microbiology Laboratory, National Research Institute for Chemical Technology, Zaria for further processing.

Isolation and characterization of A. niger from soil

Soil sample was air-dried at room temperature for 48 hr. The dried soil samples were processed to remove stones and plant residues. Serial dilution was carried-out on the soil sample into 5folds and was inoculated untu petri dishes which contain Sabouraud Dextrose Agar (Titan Biotech India) and incubated (Memmert incubator) at 28±2°C for 7 days. Discrete colonies with morphological characteristics of *A. niger* was sub-cultured to obtain pure isolates.

Pure isolates were identified based on cultural macroscopy and microscopic characterization using a standard procedures as described by Cheng *et al*., 2010 [9,10] and the Pure isolates was preserved in slants containing SDA at 4^{O}C.

Characterizing of Aspergillus niger for cellulose production

Aliquot of 0.1 ml of 10^{-5} of the solution was spread on sterile solidified isolation medium consisting of CMC 1 % (w/v), peptone 1% (w/v), yeast extract 1 % (w/v) and agar-agar (1.5 % (w/v). After incubation of the plates at 30°C for 5 days, the plates will be flooded by with 1°C Congo red dye solution for 15 min and drained.
The plates were flooded with 1.5% NaCl solution for 15 min and drain, observation for zone of clearances which is accounts for the ability to digest cellulose thus indicate the presence of cellulase. Clearance around growth of isolate was represented as cellulase production. The diameter zone of clearance was measured at five different locations and the mean was used to represent cellulase activity of the organism. The colony with the largest zone of clearance was sub cultured on fresh sterile sabouraud dextrose agar (Titan biotech India) for further studies. Based on its cellulose hydrolyzing ability, the isolate was selected for use and will be maintained on SDA slants at 4°C.

Isolation and characterizing of S. cerevisiae for glucose fermentation

Saccharomyces cerevisiae was isolated from fermented *burukutu*. The sample was serially diluted in 10 fold and 1 ml was inoculated unto Yeast extract peptone Glucose (YEPG) medium. Discrete colonies were sub-cultured on YEPG agar plates and stored in slants for further examination. Identification of *Saccharomyces* will be based on cultural, microscopic tests where the morphology of the colonies was noted in terms of shape, size, texture, elevation and margin.

Screening of S. cerevisiae using stress tolerance tests

Ethanol tolerance of yeast strains was tested by inoculating 5% broth culture of each strain in Erlenmeyer flasks with YEPG broth containing 4, 8, 12, 16, to 20% alcohol (v/v) in triplicates. After inoculation, flasks were incubated at 30°C for 48 hr. Sample was taken every 24 hr and optical density was recorded at 600 nm on Spectrophotometer (Hach). All experiments was carried out in triplicates and mean values will be considered.

Mechanical/physical pretreatment of substrates (control)

The corn cob collected was sun dried to reduce the moisture content and milled to reduce the particle size to 300 µm mesh size. This process increase its surface area there by enhanced the access of cellulase to the biomass surface and increased the conversion of cellulose [11]. 100 g of the milled corn cob was weighed into three 500ml conical flasks respectively for further hydrolysis.

Chemical pre-treatment using dilute sulphuric acid

Four percent (4%) sulphuric acid was prepared, 100 g powdered/milled corn cob was dispensed into a 500 ml Erlenmeyer flask and 200 ml of the 4% sulphuric acids was added to it and allowed to stand for 2 min. 400 ml distilled water was poured into a 1000 ml conical flask and the corn cob plus acid mixture was dispensed into the flask.

The pH was adjusted to 6.0 by adding water to the mixture. When the water does not increase the pH to 6.0, 2% NaOH was added to the solution with a dropper until the pH gets to the desired range [7]. This procedure was carried out in triplicates. The mixture was refrigerated at 4°C until further hydrolysis.

Physicochemical pretreatment using steam explosion

This process was carried out in an autoclave (portable express equipment) whereby 100 g of the grinded/milled corn cob was dispensed into 500 ml Erlenmeyer flask and autoclaved at 121°C for 30 mins. The substrates were allowed to cool and kept refrigerated until further hydrolysis. The high pressure and consequently high temperature applied on the corn cob help to degrade hemicellulose and delignify the substrate and this procedure was carried out in triplicates [12].

Inoculum preparation

A spore suspension containing 2.5 x 10^6 spores of *A. niger* and cell suspension of 3.0 x 10^8 cells/ml *Saccharomyces cerevisiae* inoculum was prepared using McFarland standard 1 to serve as inoculum [12].

Fermentation procedure

The fermentation medium to be used was supplemented with Chloramphenicol. It was

prepared and dispensed in 9 labeled 1000 ml conical flasks together with the products of pre-treated corn cob respectively. The flasks were sterilized in an autoclave and inoculated with 10 ml of the inoculum. The flasks were sealed with the aid of an adhesive tape and incubated at 500 rpm on shaker (mains stuart flask) for the period of 4 days. Bottles were removed at 24 hr intervals to determine the quantity of ethanol produced.

Determination of ethanol concentration

Thirty millilitres (30 ml) aliquot from each flask was taken after thorough shaking of the flask in order to homogenise the content at 24 hr interval during fermentation. It was used for the analysis of ethanol concentration [12]. The solution was centrifuged at 1500 rpm for 3 min. The cell free supernatant recovered was distilled [11].

Distillation

It was carried out using a distillation apparatus. The fermented liquid was transferred into a round bottom flask and placed on a heating mantle fixed to a distillation column enclosed in a running tap water. Another flask was fixed to the other end of the distillation column to collect the distillate at 78^0C (Standard temperature for ethanol production) for 2 hrs. Ethanol concentration was determined by measuring its specific gravity after distillation on a rotary evaporator using specific gravity bottle. The distillate was used to determine the ethanol concentration at 27°C as follows: A clean and dry gravity bottle of capacity 50ml was weighed with its stopper. It was filled with distilled water equal to the volume of the distillate to be measured. The excess water was wiped with a cloth, and then reweighed. The bottle was emptied and dried. It was then filled with ethanol sample and reweighed and the specific gravity was calculated.

Result and discussion

Table 1. Shows the result of ethanol tolerance profile of *saccharomyces cerevisiae strains* which were screened at 4%, 8%, 12%, 16%, and 20% ethanol concentrations. Strain1(S_1) was found to yield optical density of 0.21 at 20% of the ethanol concentrations, and as such it's was the most ethanol tolerant among all and this result is in agreement with that obtained by Tanaka *et al*., 2006 [18], which they reported that the most frequently used microbe has been yeast and among the yeasts, *Saccharomyces cerevisiae*is the most preferred strain since it can tolerate ethanol concentration as high as 20% in the fermentation medium [3].

Table 1. Ethanol tolerance profile of *Sacharomyces cerevisiae* strains

Strain	4% Ethanol	8% Ethanol	12% Ethanol	16% Ethanol	20% Ethanol	Blank
Organism		Wavelength (nm)				
S_1	1.05±0.07	0.92±1.30	0.58±0.82	0.17±0.24	0.21±0.29	1.17±0.24
S_2	1.40±0.57	1.83±1.17	0.41±0.58	0.23±0.32	0.19±0.26	1.42±0.60
S_3	1.48±0.67	0.13±0.18	0.29±0.40	0.11±0.15	0.10±0.14	1.79±1.11
S_4	1.67±0.94	1.34±0.48	0.11±0.16	0.11±0.15	0.09±0.13	1.82±1.13
S_5	1.50±0.71	0.84±1.19	0.13±0.18	0.10±0.14	0.09±0.13	1.50±0.71

Table 2. Shows the result of Bioethanol concentration generated after various pre-treatment methods. Dilute-acid pre-treatment yielded significantly higher ($p<0.05$) ethanol concentration of about 25.03% (w/v), than other pre-treatment methods due to high amount removal of hemicelluloses and lignin, also increases the accessible surface area for enzymes and decrystallize cellulose. Substrate concentration with Physical Pre-treatment released the least ethanol concentration of about 5.99 % (w/v) , which was reported that this type of pre-treatment(physical) becomes economically unfeasible for full-scale production according to Cheng *et al*., 2002 [9,10], while substrate concentration with Steam explosion pre-treatment released the average ethanol concentration and was recorded as 15.41% (w/v) due to the partial hemicelluloses hydrolysed and the lignin matrix disrupted by explosive decompression. Which is in agreement with Chandel *et al*., 2007 [6], also reported limitations of steam explosion include destruction of a portion of the xylan fraction, incomplete disruption of the lignin- carbohydrate matrix and generation of compounds that may be inhibitory to microorganisms used in fermentation processes.

The values obtained above are means ± S.D (Standard Deviation). Then the means having different superscripts are statistically different (p-value <0.05), as such the p-value obtained is 0.02. Which shows that alongside with the analysis there's significant differences between the group of the pre-treatment options. As such dilute-acid pre-treatment has high concentration compared to Steam explosion, and then Steam explosion to physical pre-treatment.

Table 2. Bioethanol concentration generated after various Pre-treatments

Pretreatments	Bioethanol Yield (%, w/v)
Dilute Acids	$25.03^{a} \pm 5.59$
Steam Explosion	$15.41^{b} \pm 2.67$
Physical (Control)	$5.99^{c} \pm 1.31$

Table 3 revealed the result of wavelength of hydroxyl groups present in the fermented end-product. The presumptive test carried after fermentation of the corncob to determine the present of the –OH group in the fermented product. The functional group peak gives a wavelength of about 3325 cm^{-1} for dilute-acid pretreatment fermented product. In-addition determination of functional group analysis clarifies the evaluation of the efficient pretreatment methods that is, there's high amount of alcohol group (OH) in the dilute-acid pretreatment method compared to others, and was used to strongly support the above statement that dilute-acid pretreatment method yielded high amount of ethanol concentration on Corncob. This result was compared with that obtained by Yoo *et al*., 2011 [13], 69.2% cellulose to glucose conversion was achieved by dilute acid hydrolysis pretreatments, respectively. Yoo *et al.,* 2011 [13], analyzed the technical and economic competitiveness of dilute acid hydrolysis and steam explosion and physical pretreatment (control) for cellulosic ethanol production.

Table 3. Wavelength of hydroxyl groups present in the fermented end product

Pretreatment	Wavelength (cm^{-1}) OH (3300- 3400)
Physical pretreatment	3302.24
Steam explosion pretreatment	3309.00
Dilute acid pretreatment	3325.00

Key: O-H= hydroxyl functional group.

Conclusions

In conclusion, among the pre-treatment methods used, dilute acid method yielded highest bioethanol concentration and most promising compared to steam explosion and grinding methods. The increasing demands for energy and the shrinking energy resources, the utilization of lignocelluloses biomass for the production of bioethanol offers a renewable alternative. Apart from bioethanol's, other value-products such as fermentable sugars, organic acids, solvents and drink softeners etc. may also be produced from lignocelluloses biomass using appropriate technologies. Theoretically this is quite possible; however technologically it is not an easy task because of various technological gaps. However, *Saccharomyces cerevisiae* and *Aspergillus niger* has been known in the production of certain enzymes required for the fermentation of cellulosic compounds into bioethanol in sufficient quantities As such various methods of pre-treatment was carried alongside with for the effective degradations of the lignocelluloses biomass (i.e Dilute-acid, Steam explosion and Grinding). Whereby Dilute-acid pre-treatment shows highest yield of ethanol of about 25.03^{a}±5.59, then Steam explosion with 15.41^{b}±2.67 and Physical pre-treatment being the least and recorded as 5.99^{c}±1.31 for releasing the fermentable sugars. However, no single cost effective and efficient technology is currently available to meet the challenges of large-scale utilization of lignocelluloses biomass therefore we suggest here and integrated approach including efficient bioreactor design selection and optimization of physical and chemical conditions for several organisms maybe used under co-cultivation conditions.

Conflicts of interest

No conflict of interest.

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